

April 4, 1950.

Dear Max:

I think that a separate detailed account of your pedigree technique and its potentialities would be a splendid idea. Among other things, it saves my name being on a paper containing important techniques that I had nothing to do with. It would also be useful to have the two papers concurrent.

Differential X-ray killing of haploid and diploid yeast (to which you made some sort of oblique reference) may have little bearing on the bacterial work, inasmuch as the total nuclear content is not controlled in the latter. There is also some old work of Whiting's on differentials between n and $2n$ *Habrobracon*.

The latest dope on pH effects: Acetate buffer, pH 4, rapidly kills diploid cells which have been grown on Davis-lactose. (Decimal reduction time about 5 minutes.) The effect can also be demonstrated at somewhat higher pHs, which K-12 will reach if grown on enough sugar (1%) and inadequate buffer. The main point is that the survivors of the acetic acid killing are decidedly enriched in haploids. I suspect that this is at least partly a selective effect, killing the diploids preferentially?, and the enrichment is nowhere near as dramatic, for small killing, as it is with UV, mustard, etc., where there is no question of selection. However, this point militates against using media on which low pHs will be attained, if this can be avoided. This may account for the rather unsatisfactory results, in general, with EMS.

The general effect can be duplicated by comparing growth of H-226 with and without glucose. Davis' medium has enough buffer to neutralize the acid from about .1-.2% sugar. With N-Z-Case as a secondary carbon source, and to ~~minimize~~ eliminate selective advantage for prototrophs, the proportion of diploid cells is much less if 1% glucose is added, although the final level of growth is about the same. In the absence ~~mgf~~ glucose, but in Davis' + N-Z-Case, adjusted to pH 5.9 with acetic acid, the final proportion of diploid cells was only 20%, with about 2/7 generations of growth. This medium at pH 5.0 did not allow the initiation of growth; in another experiment using D' (= Davis') .1% glucose at pH 4.9, slight growth was obtained, all segregated. I used rather too large an inoculum (.5 %) to get the most drastic effects in these experiments, but they make it clear that isolations are best made to medium without much sugar. A D' 0.1% lactose + 1% NZCase might be best.

This is not to say that I had any difficulty in your last set of isolations. Your concern that you had segregant cells to start with was groundless: each one was diploid!

All isolations were Lac v (and Mal+, probably v) except:

A23-24 Lac- Mal- B-61 L- M+ F21 L-M~~+~~

As predicted, F2 progenies were sterile. However, the tubes were faintly turbid, and some growth may yet occur. Is it possible that this might be a contaminant, slow growing culture?

Except by adding to estimations of segregation frequency and pattern, these data are in line with previous. I am hoping that 1) an autogamous change can be picked up, and 2) a crossover type to check whether the sib diploids have changed their crossover patterns.

Quite a few more hemizygosity tests: Other Mal- diploids from the same cross as H-226 are hemizygous for Mal-; other "partial segregant" Mal- from H-226 are homozygous for Mal .!. There are, then, too distinct peculiar phenomena.

I'll send back the vials as soon as possible. Please let me know of anything else we can do here,

Sincerely,

Joshua Lederberg